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Immunohistochemical distribution of platelet-derived growth factor-C and platelet-derived growth factor receptor-α in small intestine of rats treated with capsaicin

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Abstract: The purpose of this study was to examine the effect of capsaicin administration immunohistochemically on platelet-derived growth factor (PDGF)-C and platelet-derived growth factor receptor (PDGFR)-α release in the small intestine tissues of rats. Rats were divided into three groups: group 1 (subcutaneous drug administration), group 2 (oral drug administration), and group 3 (subcutaneous sham administration). Histometric measurements and counts were performed in order to determine whether there was a difference between groups in terms of height of villi, depth of crypts, and goblet cell count in small intestine. The small intestine was examined by Crossman's triple staining method and streptavidin-biotin peroxidase complex method. As a result of statistical analysis, it was found that body weight increased in group 3, while it decreased in groups 1 and 2. Capsaicin administration increased height of villi, depth of crypts, and count of goblet cells located on villi and crypts. Immunohistochemical examinations revealed that capsaicin administration increased PDGF-C and PDGFR-α release. It was concluded that capsaicin has a stimulatory effect on growth and is an enhancer for PDGF-C and PDGFR-α release.

Key words: Capsaicin, PDGF-C, PDGFR-α, small intestine

1. Introduction

Capsaicin belongs to the genus *Capsicum* of the family Solanaceae. Capsaicin, from *Capsicum annuum* (1), is the colorless, odorless, hydrophobic, and active substance of chili pepper (2). Capsaicin has been reported to have effects on the gastrointestinal, cardiovascular, and respiratory systems (3) and on lipid metabolism (4). The effect of capsaicin, which is a studied subject in numerous fields today, varies based on the applied dose, organ, and exposure time (5).

Being a member of the family of growth factors, platelet-derived growth factor (PDGF) has important functions for growth and development (6). PDGF consists of five different homodimer and heterodimer subunits: PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC, and PDGF-DD (7). PDGF-C has protein properties and is one of the recently discovered members of the PDGF family (8). PDGF-C has significant functions such as in cell reproduction, wound healing, organ remodeling, and development of both embryonic and adult tissues (6).

Platelet-derived growth factor receptor-α (PDGFR-α) is a member of the PDGF receptor family. (7). PDGFR-α, which is located on the fourth chromosome (9), has protein properties with 170 kDa molecular weight and 1089 amino acids (7). PDGFR-α binds and activates PDGF dimers such as PDGF-AA, PDGF-BB, and PDGF-CC (10).

This study was conducted in order to investigate the effect of capsaicin, which is known to have a long-standing history and a wide area of use, especially spices and the medical field, on the distribution of PDGF-C and PDGFR-α in the small intestines of capsaicin-administered rats.

2. Materials and methods

2.1. Animals

Tissue samples were collected in compliance with an approved Kafkas University Institutional Animal Care and Use Committee protocol (KAÜ-HADYEK 11.06.2009/04).

Thirty 50-day-old female Sprague Dawley rats were randomly divided into three groups: group 1 (n = 10) (subcutaneous drug administration), group 2 (n = 10) (oral drug administration), and group 3 (n = 10) (subcutaneous sham administration). Rats were housed in a continuously ventilated room at a mean temperature of 22 ± 2 °C with a lighting period of 12 h dark and 12 h light. Animals were fed standard rodent chow (Bayramoğlu, Erzurum, Turkey) and water ad libitum. The amount of capsaicin used in

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our study was based on studies conducted by Moran et al. (11) and Tütüncü (12). For the rats in group 1, 1 mg/kg capsaicin (cat no. M 2028, Sigma-Aldrich, Germany) was dissolved in 10% ethanol and mixed with 1% Tween (cat no. M 8170772100, Merck, USA) and 80% distilled water. Capsaicin solution was freshly prepared according to daily body weights of the rats and injected subcutaneously with an insulin injector at the same time every day for a week. For the rats in group 2, 1 mg/kg capsaicin was dissolved in 10% ethanol and mixed with 1% Tween and 80% distilled water. Capsaicin solution was freshly prepared according to daily body weights of the rats and added to their drinking water. For the rats in group 3, a solution of 10% ethanol, 1% Tween, and 80% distilled water was injected subcutaneously as described previously. All rats were weighed daily before injection and oral administration of solutions. After 1 week, all rats were sacrificed by cervical dislocation method under diethyl ether anesthesia and small intestine samples (duodenum, jejunum, and ileum) were taken.

2.2. Histomorphometric evaluations and statistical analysis

In order to determine whether there was a difference among groups in terms of villus height, crypt depth, and goblet cell count in the small intestine, histometric measurements and counts were performed by means of ocular micrometer adjusted to the research microscope (Olympus CX21, Japan). Villus height, crypt depth, and goblet cell count present in villi and crypts were determined in five villi chosen randomly from six sections taken serially from the duodenum, jejunum, and ileum of each animal. Villus height was measured from the tip of the villus to the villus-crypt junction by using 10× objective, crypt depth was determined by measuring the distance between edges of crypts with 40× objective, and goblet cell count in villi and crypts was directly counted with 10× and 40× objectives. SPSS 15.0 was used in order to statistically compare the data on body weight measurements, villus height, crypt depth, and goblet cell count among groups. Possible differences were determined using ANOVA and t-tests. The confidence interval in statistical analysis was determined as 0.05.

2.3. Histological procedure

Samples were fixed in 10% formaldehyde for 24 h, and then routine procedures were applied and samples were embedded in paraffin. Serial sections of 5 µm thick were sliced from paraffin-embedded blocks and Crossman's triple staining method was used to demonstrate the general structure of the small intestine.

2.4. Immunohistochemical procedure

For immunohistochemistry, the streptavidin-biotin peroxidase complex method was applied to 5-µm-thick sections. After deparaffinizing and rehydrating the sections, endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 20 min. Sections then were washed in 0.01 M phosphate buffered saline (PBS) solution, pH 7.4. Sections were incubated with polyclonal goat anti-PDGF-C antibody (sc-18228, Santa Cruz Laboratories, Santa Cruz, CA, USA) diluted 1:50 in PBS and polyclonal rabbit anti-PDGFR-α antibody (sc-338, Santa Cruz Laboratories) diluted 1:400 in PBS. Sections were incubated with biotinylated goat antirabbit and rabbit antigoat IgG for 30 min and peroxidase-conjugated streptavidin (1:300) (P0397; Dako Corp., Carpinteria, CA, USA) for 30 min. 3,3′-Diaminobenzidine tetrahydrochloride (0.5 mg/mL; Dako Corp.) was used as chromogen followed by hematoxylin counterstaining. Sections were mounted with ImmunoMount and examined by light microscope (Olympus BX51, Tokyo, Japan). Rabbit serum without primer antibody served as the negative control. Immunostaining intensity of PDGF-C and PDGFR-α immunoreactive cells were evaluated in four different cross-sections and by two different researchers. Immunoreactive cells were categorized as having (-) negative, (+) very slight, (++) slight, (+++) moderate, (++++) intensive, or (+++++) very intensive immunolabeling.

3. Results

3.1. Body weight results

The statistical results showed that while body weights of groups 1 and 2 decreased compared to the first day, there was a significant increase ($P < 0.05$) in terms of body weight in group 3. In light of the statistical data, capsaicin administration was determined to cause a decrease in body weight (Table 1).

3.2. Villus height results

When all the groups were compared in terms of villus height, there was a significant difference among them ($P < 0.05$). An increase in villus height was observed in group 1 compared to other groups (Table 2).

3.3. Crypts depth results

When all the groups were compared in terms of crypts depth, there was a significant difference among them ($P < 0.05$). An increase in crypts depth was observed in groups 1 and 2 (Table 3).

3.4. Count of goblet cells located on villi

When all the groups were compared in terms of count of goblet cells located on villi, there was a significant difference among them ($P < 0.05$). An increase in count of goblet cells located on villi was observed in groups 1 and 2 (Table 4).

3.5. Count of goblet cells located on crypts

When all the groups were compared in terms of count of goblet cells located on crypts, there was a significant

Table 1. The mean body weight of rats in all three groups on the first day and day 14 (g).

Group	Number (n)	AM ± SD (first day)	AM ± SD (day 14)	SIG	P
Group 1	10	163.00 ± 10.79	147.80 ± 9.15	0.025	**
Group 2	10	142.20 ± 12.59	126.40 ± 12.08	0.041	**
Group 3	10	129.30 ± 9.70	163.40 ± 14.83	0.001	**

AM: Arithmetic mean; SD: standard deviation; SIG: significance, **P < 0.05.

Table 2. Comparison of villus height among groups (μm).

Group	Number (n)	Duodenum	Jejunum	Ileum	P
Group 1	300	73.96 ± 7.45^{a}	59.46 ± 6.02^{a}	54.27 ± 6.13^{a}	**
Group 2	300	69.15 ± 8.49^{b}	54.37 ± 6.70^{b}	49.27 ± 6.12^{b}	**
Group 3	300	60.02 ± 5.15^{c}	46.59 ± 4.98^{c}	42.57 ± 5.39^{c}	**

Different superscripts in the same column indicate significant differences between groups, **P < 0.05.

Table 3. Comparison of crypt depth among groups (μm).

Group	Number (n)	Duodenum	Jejunum	Ileum	P
Group 1	300	52.99 ± 8.65^{a}	62.30 ± 7.99^{a}	64.02 ± 6.35^{a}	**
Group 2	300	48.62 ± 7.20^{b}	59.52 ± 8.49^{b}	61.96 ± 7.99^{b}	**
Group 3	300	42.88 ± 7.29^{c}	48.44 ± 5.49^{c}	50.22 ± 4.08^{c}	**

Different superscripts in the same column indicate significant differences between groups, **P < 0.05.

Table 4. Comparison of count of goblet cells located on villi among groups.

Group	Number (n)	Duodenum	Jejunum	Ileum	P
Group 1	300	28.74 ± 5.45^{a}	30.81 ± 3.88^{a}	36.69 ± 5.87^{a}	**
Group 2	300	23.78 ± 4.07^{b}	25.62 ± 2.79^{b}	33.64 ± 7.49^{b}	**
Group 3	300	18.72 ± 3.44^{c}	20.44 ± 2.80^{c}	25.70 ± 4.73^{c}	**

Different superscripts in the same column indicate significant differences between groups, **P < 0.05.

difference among them (P < 0.05). An increase was observed in count of goblet cells located on crypts especially in group 1 compared to groups 2 and 3 (Table 5).

3.6. Histological results

Normal histological results were obtained and no difference was observed in the histological structure of rats in all three groups (Figure 1).

3.7. Immunohistochemical results

Specific PDGF-C and PDGFR-α reactions were observed in small intestine tissues of all groups.

3.7.1. PDGF-C immunoreactivity in duodenum

Slight cytoplasmic reaction was observed in villus epithelial cells of all groups. While intensive nuclear reaction was observed in goblet cells of groups 1 and 2, moderate nuclear reaction was remarkable in goblet cells

Table 5. Comparison of count of goblet cells located on crypts among groups.

Group	Number (n)	Duodenum	Jejunum	Ileum	P
Group 1	300	11.87 ± 2.54[a]	15.12 ± 2.63[a]	20.59 ± 5.08[a]	**
Group 2	300	9.34 ± 1.20[b]	11.50 ± 2.08[b]	16.19 ± 3.88[b]	**
Group 3	300	6.25 ± 1.50[c]	7.80 ± 1.63[c]	11.74 ± 3.50[c]	**

Different superscripts in the same column indicate significant differences between groups, **P < 0.05.

of group 3. Slight nuclear reaction was determined in crypt epithelial cells of all groups. Cytoplasmic reaction in vascular endothelial cells was very intense in group 1, slight in group 2, and very slight in group 3. While very slight reaction was observed in smooth muscle cells in group 1, no reaction was determined in smooth muscle cells in groups 2 and 3 (Figure 2).

3.7.2. PDGF-C immunoreactivity in jejunum

While moderate cytoplasmic reaction was observed in villus epithelial cells of groups 1 and 2, very slight cytoplasmic reaction was present in villus epithelial cells of group 3. While very intensive nuclear reaction was present in goblet cells of groups 1 and 2, slight nuclear reaction was observed in goblet cells of group 3. It was observed that while rats in groups 1 and 2 had moderate cytoplasmic reaction in crypt epithelial cells, group 3 had very slight cytoplasmic reaction. While intensive cytoplasmic reaction was observed in vascular endothelial cells in groups 1 and 2, slight cytoplasmic reaction was present in group 3. It was remarkable that while very slight reaction was observed in smooth muscle cells in group 1, there was no reaction in groups 2 and 3 (Figure 3).

3.7.3. PDGF-C immunoreactivity in ileum

While villus epithelial cells of group 1 had slight cytoplasmic reaction, very slight cytoplasmic reaction was identified in villus epithelial cells of groups 2 and 3. There

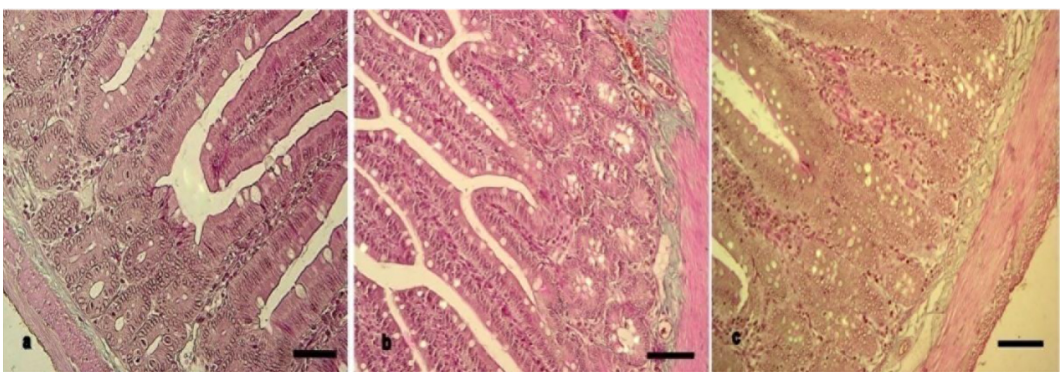

Figure 1. Rat ileum (a), rat jejunum (b), rat duodenum (c). Triple staining. Bar: 100 µm.

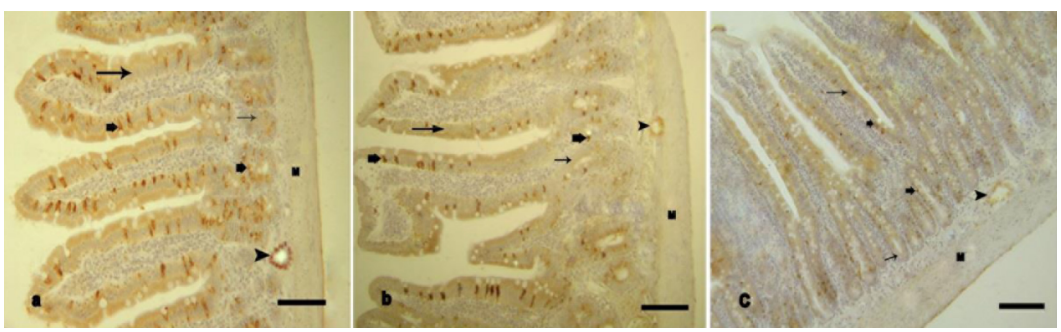

Figure 2. PDGF-C immunoreactivity in rat duodenum: group 1 (a), group 2 (b), group 3 (c), villus epithelial cell (long arrow), goblet cells (thick arrows), crypt epithelial cell (short arrow), endothelial cell (arrow head), smooth muscle cell (M). Immunohistochemistry. Bar: 200 µm.

was very intensive nuclear reaction in goblet cells of group 1, intensive nuclear reaction in those of group 2, and slight nuclear reaction in those of group 3. Crypt epithelial cells were observed to have slight cytoplasmic reaction in group 1 and very slight cytoplasmic reaction in groups 2 and 3. Vascular endothelial cells had very intense reaction in group 1, moderate reaction in group 2, and slight reaction in group 3 (Figure 4).

3.7.4. PDGFR-α immunoreactivity in duodenum

Slight cytoplasmic reaction was observed in villus epithelial cells of all groups. While the nuclear reaction observed in goblet cells was very intensive in groups 1 and 2, it was slight in group 3. Crypt epithelial cells of all groups had slight cytoplasmic reactions, similar to villus epithelial cells. Reaction in vascular endothelial cells was very intensive in groups 1 and 2 and slight in group 3. Reaction in smooth muscle cells was slight in group 1, moderate in group 2, and very slight in group 3 (Figure 5).

3.7.5. PDGFR-α immunoreactivity in jejunum

While moderate cytoplasmic reaction was determined in villus epithelial cells of group 1, very slight cytoplasmic reaction was observed in the villus epithelial cells of groups 2 and 3. Goblet cells had very intensive nuclear reaction in groups 1 and 2 and slight nuclear reaction in group 3. It was determined that groups 2 and 3 had very slight cytoplasmic reaction in crypt epithelial cells, while group 1 had moderate cytoplasmic reaction. Reaction in the vascular endothelial cells was intensive in group 1, moderate in group 2, and slight in group 3 (Figure 6).

3.7.6. PDGFR-α immunoreactivity in ileum

It was found that while villus epithelial cells of group 1 had moderate cytoplasmic reaction, the reaction was slight in those of group 2 and very slight in those of group 3. Reaction in goblet cells was determined to be very intensive in groups 1 and 2 and slight in group 3. Crypt epithelial cells of group 1 had moderate cytoplasmic reaction, those of group 2 had slight cytoplasmic reaction, and those of group 3 had very slight cytoplasmic reaction. Regarding vascular endothelial cells, while group 1 had very intensive reaction, group 2 had intensive reaction and group 3 had slight reaction (Figure 7).

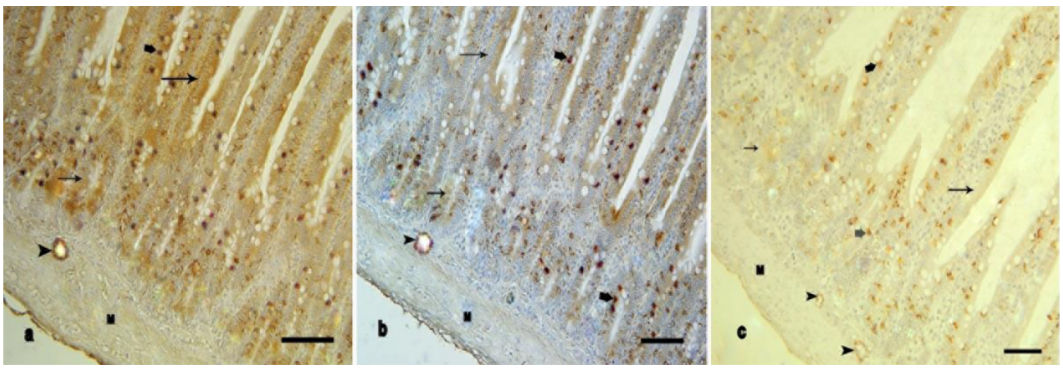

Figure 3. PDGF-C immunoreactivity in rat jejunum: group 1 (a), group 2 (b), group 3 (c), villus epithelial cell (long arrow), goblet cells (thick arrows), crypt epithelial cell (short arrow), endothelial cell (arrow head), smooth muscle cell (M). Immunohistochemistry. Bar: 200 µm.

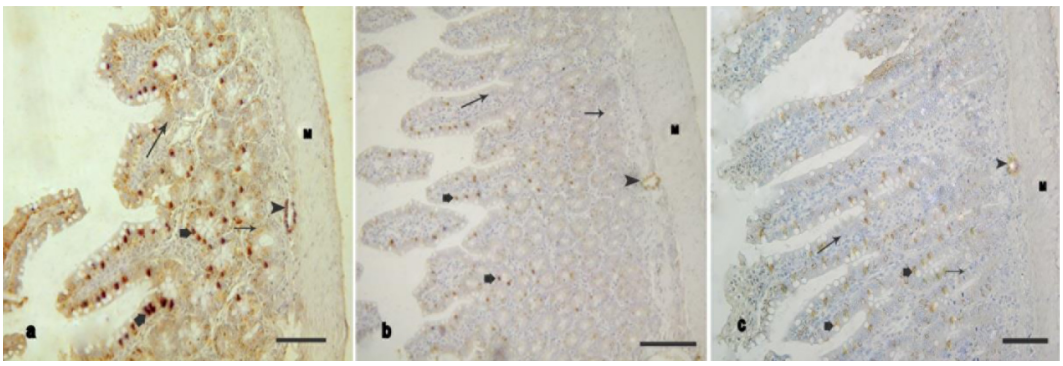

Figure 4. PDGF-C immunoreactivity in rat ileum: group 1 (a), group 2 (b), group 3 (c), villus epithelial cell (long arrow), goblet cells (thick arrows), crypt epithelial cell (short arrow), endothelial cell (arrow head), smooth muscle cell (M). Immunohistochemistry. Bar: 200 µm.

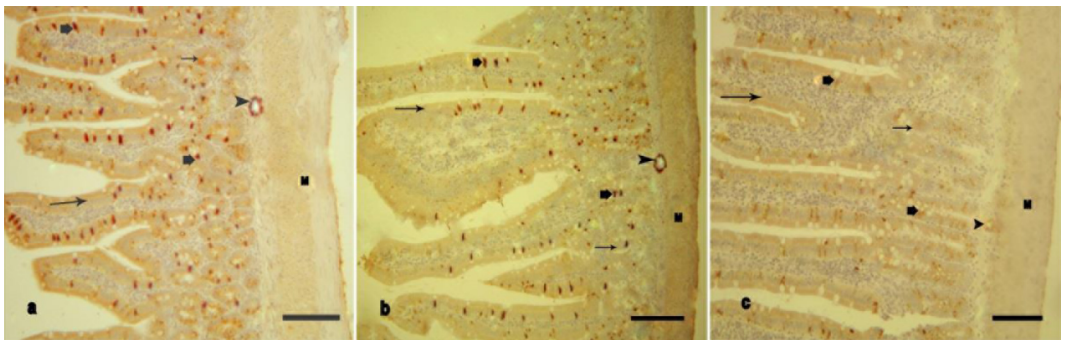

Figure 5. PDGFR-α immunoreactivity in rat duodenum: group 1 (a), group 2 (b), group 3 (c), villus epithelial cell (long arrow), goblet cells (thick arrows), crypt epithelial cell (short arrow), endothelial cell (arrow head), smooth muscle cell (M). Immunohistochemistry. Bar: 200 µm.

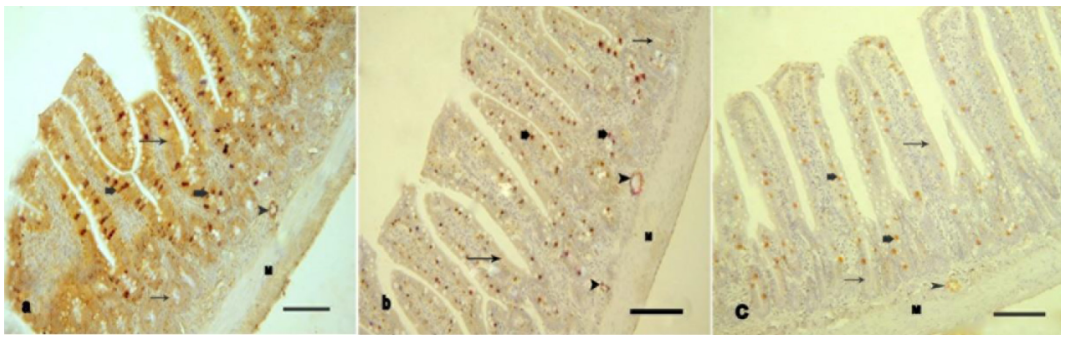

Figure 6. PDGFR-α immunoreactivity in rat jejunum: group 1 (a), group 2 (b), group 3 (c), villus epithelial cell (long arrow), goblet cells (thick arrows), crypt epithelial cell (short arrow), endothelial cell (arrow head), smooth muscle cell (M). Immunohistochemistry. Bar: 200 µm.

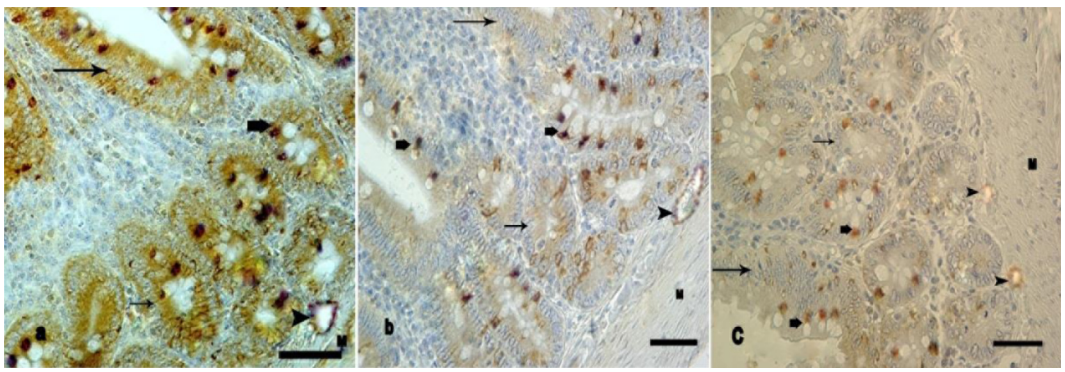

Figure 7. PDGFR-α immunoreactivity in rat ileum: group 1 (a), group 2 (b), group 3 (c), villus epithelial cell (long arrow), goblet cells (thick arrows), crypt epithelial cell (short arrow), endothelial cell (arrow head), smooth muscle cell (M). Immunohistochemistry. Bar: 50 µm.

4. Discussion

It was reported that the effects of capsaicin on body weight change are especially based on lipid metabolism (4,13,14). Capsaicin was asserted to have this effect by decreasing lipid absorption from the intestine, increasing functions of glucose-6-phosphate and lipoprotein lipase and thus decreasing serum triglyceride levels, weight of adipose tissue, and lipid peroxidation (4,13). It was suggested that the decrease in lipid level was associated with capsaicin providing the release of catecholamines from the adrenal medulla and increasing the energy and lipid metabolism due to the fact that it stimulates the central nervous system.

Capsaicin is used in the treatment of obesity because of these effects (15). Comparing all groups in this study, there was a decrease in body weights of groups 1 and 2 and an increase in body weights of rats in group 3. Capsaicin was determined to decrease body weight in consideration of the statistical data.

Because of effects on growth and development in the intestine, in a study conducted on this subject, an increase was reported in villus height in the small intestine of animals fed with a plant extract mixture consisting of carvacrol, cinnamaldehyde, and capsaicin. It was found here that villus height of intestines of capsaicin-administered rats in groups 1 and 2 increased, in parallel with the information in the literature (16).

The small intestine, especially crypts and villi of the absorptive epithelium, plays a significant role in nutrient digestion and assimilation. An elongation of the villus increases the surface area for nutrient absorption. The crypt can be regarded as the villus factory, and a deeper crypt indicates faster tissue turnover and a high demand for new tissue. The increase in number of goblet cells was accepted as an indicator of absorption and digestive capacity in enterocytes (17). Capsaicin was suggested to increase the amount of substances absorbed in intestines by increasing the number of goblet cells and providing a protective effect in the digestive system by increasing mucous production in the stomach (1,17). Capsaicin was also stated to increase the absorption of substances in the small intestine (18). Comparing all groups in this study, villus height, crypt depth, and number of goblet cells in both villi and crypts increased in small intestine tissues of groups 1 and 2. In light of these results, capsaicin is thought to increase both absorption of substances by increasing the number of goblet cells and to have a protective feature against physical and chemical effects by increasing mucous production.

Growth factors have significant roles on the development of the small intestine in embryonic and adult periods. However, there is limited information regarding this subject (19). It was reported that both PDGF-A and PDGFR-α were necessary for development of the gastrointestinal system, and release of these factors was necessary in villus and crypt epithelial cells, the submucosa, and muscles. It was stated that while PDGFs are released especially from epithelial cells, PDGFR-α is released from cells of muscles and connective tissues along with epithelial cells (20). Even though expression details of PDGF/PDGFR-α signals could not be explained in adult gastrointestinal tissue, PDGFR-α is released at high levels in small intestine tissue of adult rats (21). Our study determined that PDGF-C and PDGFR-α immunoreactivity was present in villus and crypt epithelial cells, goblet cells, vascular endothelial cells, and smooth muscle cells in the tissue of the small intestine.

No information was found about the effects of capsaicin on PDGF-C and PDGFR-α release. However, it was reported to have an increasing effect on release of various growth factors such as IGF-I (22), EGF (23), and TGF (24). A study conducted on humans and animals reported that capsaicin administration increased development of skin and hair by stimulating release of IGF-I, a growth factor. Release of PDGF-C and PDGFR-α in the small intestine (duodenum, jejunum, and ileum) of rats in groups 1 and 2 increased in this study, in parallel with other studies mentioned (23–25).

The present study concluded that while body weight increased in group 3, it was decreased in groups 1 and 2. Capsaicin administration increased villus height, crypt depth, and goblet cell count in the small intestine and showed an increasing effect on growth by increasing PDGF-C and PDGFR-α release in numerous cells such as villus and crypt epithelial cells, goblet cells, vascular endothelial cells, and smooth muscle cells.

Acknowledgment
The present study was summarized from a PhD thesis.

References

1. Eshbaugh WH. A nomenclatural note on the genus *Capsicum*. Taxon 1968; 17: 51–52.
2. Singh S, Natarajen K, Agrawall BB. Capsaicin (C8-methyl-N-vanillyl-6-nonenamide) is potent inhibitor of nuclear transcription factor-kappa B activation by diverse agents. J Immunol 1996; 157: 4412–4420.
3. Surh YJ, Lee SS. Capsaicin, double-edged sword: toxicity, metabolism, and chemo preventive potential. Life Sci 1995; 56: 1845–1855.
4. Kawada T, Sakabe S, Watanabe T, Yamamato M, Iwai K. Some pungent principle of spices causes the adrenal medulla to secrete catecholamine in anesthetized rats. P Soc Exp Biol Med 1988; 188: 229–233.
5. Kim JD, Kim JM, Pyo JO, Kim SY, Kim BS, Yu R, Han IS. Capsaicin can alter the expression of tumor forming-related genes which might be followed by induction of apoptosis of a Korean stomach cancer cell line SNU-1. Cancer Lett 1997; 120: 235–241.
6. Deuel F. Polypeptide growth factors: roles in normal. Annu Rev Cell Biol 1987; 3: 443–492.
7. Heldin CH, Westermark B. Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 1999; 79: 1283–1316.
8. Reigstad LJ, Varhauq JE, Lillehauq JR. Structural and functional specificities of PDGF-C and PDGF-D the novel members of the platelet-derived growth factor family. FEBS J 2005; 272: 5723–5741.

9. Bonthron DT, Morton CC, Orkin SH, Collins T. Platelet-derived growth factor A chain: Gene structure, chromosomal location and basis for alternative mRNA splicing. P Natl Acad Sci USA 1988; 85: 1492–1499.

10. Shure D, Senior RM, Griffin GL, Deuel TF. PDGF-AA homodimers are potent chemoattractants for fibro blast and neutrophils, and for monocytes activated by lymphocytes or cytokines. Biochem Biophys Res Commun 1994; 186: 1510–1514.

11. Moran C, Morales L, Razo RS, Apolonio J, Quiroz U, Chavira R, Dominguez R. Effects of sensorial denervation induced by capsaicin injection at birth or on day three of life, on puberty, induced ovulation and pregnancy. Life Sci 2003; 73: 2113–2125.

12. Tütüncü S. Immunohistochemical expression of VR1 in ovaries of capsaicin treated rats during development. PhD, Uludağ University, Bursa, Turkey, 2009.

13. Kawada T, Hagihara KI, Iwai K. Effects of capsaicin on lipid metabolism in rats fed a high fat diet. J Nutr 1986; 116: 1272–1280.

14. Naponitaya W. Long-term effects of capsaicin on fat absorption and the growth of the rat. Growth 1973; 37: 269–279.

15. Kim C, Robert AM, Joshua IG. Differences in the capsaicin-induced dilation of arterioles and venules in rat striated muscle. J Pharmacol Exp Ther 1995; 273: 605–610.

16. Catala P, Garcia V, Orengo J, Madrid J, Hemandez F, Corpa J, Ortega J. Effect of plant extracts and chickens location on intestine mucosal morphology and ileal apparent digestibility of broilers. In: XXII World's Poultry Congress Book of Abstracts, İstanbul, Turkey, 2004. p. 456.

17. Erdoğan D, Hatiboğlu MT, Görgün M, Ilgaz C. Özel Histoloji. 1st ed. Ankara, Turkey: Hatipoğlu Publishers; 1996 (in Turkish).

18. Udupihille M. The effect of capsaicin on the small intestinal absorption of glucose and alanine in the rat. Indian J Physiol Pharmacol 1993; 37: 59–62.

19. Booth C, Evans GS, Potten CS. Growth factor regulation of proliferation in primary culture of small intestinal epithelium. Cell Dev Biol 1995; 31: 234–243.

20. Karlsson L, Lindahl P, Heath JK, Betsholtz C. Abnormal gastrointestinal development in PDGF-AA and PDGFR-α deficient mice implicates a novel mesenchymal structure with putative instructive properties in villus morphogenesis. Development 2000; 127: 3457–3466.

21. Chan F, Liu Y, Sun H, Li X, Shang H, Fan D, An J, Zhou D. Distribution and possible role of PDGF-AA and PDGFR-α in the gastrointestinal tract of adult guinea pigs. Virchows Arch 2010; 457: 381–388.

22. Harada N, Okajima K, Arai M, Kurihara H, Nakagata N. Administration of Capsaicin and isoflavone promotes hair growth by increasing insulin-like growth factor production in mice and in humans with alopecia. Growth Horm IGF Res 2007; 17: 408–415.

23. Raybould HE. Capsaicin sensitivity and epidermal growth factor. Gut 1998; 42: 314–315.

24. Akkoç OCG. Postnatal gelişim dönemlerinde capsaicin uygulanan fare testislerinde transforming growth factor β'nın immunohistokimyasal ekspresyonu. PhD, Uludağ University, Bursa, Turkey, 2007 (in Turkish).

25. Inoue K, Koizumi S, Fuziwara S, Denda S, Denda M. Functional vanilloid receptors in cultured normal human epidermal keratinocytes. Biochem Biophys Res Commun 2002; 291: 124–129.